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In vitro antibacterial activity of *Eclipta alba* (L.) Hassk

S. Selvamani*, S. Balamurugan

Department of Botany, Annamalai University, Annamalai Nagar, India

*E-mail address: drbalu2011@gmail.com

ABSTRACT

The antibacterial activity of *Eclipta alba* against three gram positive and five gram negative bacterial strains was investigated. The fresh whole plants were collected from Chidambaram, Tamil Nadu. Petroleum ether, chloroform, ethyl acetate, acetone, methanol and aqueous extracts at different concentrations (1, 2, 5, 10 mg/ml) were used to investigate the antibacterial activity. NCCL standards were strictly followed to perform antibacterial disc susceptibility test using disc diffusion method. The extracts showed varying degree of inhibitory potential against all the tested bacteria. Methanol extract of the plant had higher inhibitory action against *Staphylococcus aureus, Bacillus subtilis*, *Proteus mirabilis* and *Pseudomonas fluroescens*.

***Keywords*:** *Eclipta alba*; Antibacterial activity; Methanol and Acetone extracts

1. INTRODUCTION

In the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unnani due to the adverse effects associated with synthetic drugs. Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances (Shankar *et al*., 2003). Plants have the capacity to synthesize a diverse array of chemicals. *Eclipta alba* is used as a tonic and diuretic in hepatic and spleen enlargement. It is also catarrhal jaundice and for skin diseases (Dalal *et al*., 2010).

The alcoholic extract of the plant has shown antiviral activity against Ranikhet disease virus (Khare, 2004). The plant is commonly used in hair oil all over India for healthy black and long hair (Roy *et al*., 2008). The fresh juice of leaves is used for increasing appetite, improving digestion (Chery, 2007) and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma (Thakur and Megni, 2005) and popularly used to enhance memory and learning (Jadhav, 2009).

Eclipta alba has a reputation as an ant ageing agent in Ayurveda (Thakur and Mengi, 2005). The plant is used as a general tonic for debility.

Externally the plant is used for inflammation (Singh *et al.*, 2008), minor cuts and burns and the fresh leaf-juice is considered very effective in stopping bleeding (Khan and Khan,

2008). Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections.

The source of coumestans-type compounds used in phytopharmaceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis (Wanger *et al.*, 1986, Scott, 1998, Thakur and Mengi, 2005). It is widely used in India as a chologuague and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder (Upadhyay *et al.*, 2001, Lal *et al.*, 2010). Vedic Guard, a polyherbal formulation is a synergistic combination of 16 medicinal plant extracts contains *Eclipta alba* as a major ingredient (Razdan *et al.,* 2008). Charaka advises taking the juice of *Eclipta alba* with honey to prevent the onset of senility, and its oil as the best medicated massage oils for rejuvenation therapies.

This plant is well documented and several *in vitro* and *in vivo* studies describe its antiageing agent and anti-hepatotoxic properties (Saxena *et al.,* 1993). The present study evaluates the potential of *Eclipta alba* extracts for their antimicrobial activity against important human pathogens.

2. MATERIALS AND METHODS

2. 1. Plant material

The whole plant of *Eclipta alba* was collected from the different places in and around area of Annamalai University, Annamalainagar campus Chidambaram, Tamil Nadu. The plant was authenticated by Dr. V. Venkatesalu, Professor and Head, Botany Wing (DDE). Where a voucher specimen was deposited.

2. 2. Extraction procedure

The plant material was washed with water and shade dried at room temperature. The dried plant materials were ground into fine powder in an electric blender and subsequently sieved for obtaining fine powder. 30 gms of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using petroleum ether, chloroform, ethyl acetate, acetone, methanol and water successively. Before extraction with the next solvent the powder was air dried to remove the adhering solvent. The extract obtained was filtered and concentrated in rotary flash evaporator. The concentrated plant extract was used for antimicrobial assays.

2. 3. Test bacteria

A total of eight bacterial species were tested in the present study. The gram positive species were *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* and gram negative species were *Escherichia coli, Proteus mirabilis, Pseudomonas fluroescens*, *Pseudomonas aeruginosa* and *Salmonella typhi*. These pathogenic strains were obtained from the Department of Medical Microbiology, Rajah Muthaiya Medical College & Hospital, Annamalai University, Annamalai Nagar. The bacterial strains were maintained on nutrient agar slants at 4 °C.

2. 4. Culture media and inoculums preparation

Nutrient agar (Himedia, India) were used as the bacterial culture medium in the bacterial assays. Loops full of all the bacterial cultures were inoculated in the 50 ml of sterile nutrient agar (NA) in 100 ml conical flask at 37 °C for 72 hrs.

2. 5. Antibacterial activity

The extracts obtained were screened for their antibacterial activity in comparison with standard antibiotic penicillin (10 mg/mL) *in vitro* by disc diffusion method using various bacterial strains (Bauer *et al*., 1966). The paper discs (6 mm diameter, Whatman No. 1 filter paper) containing 1.0, 2.0, 5.0, 10.0 mg/ml plant extracts were dried and placed aseptically on the agar surface with the help of a sterile forceps and paper discs were pressed slightly with the forceps to make complete contact with the surface of the medium (Sainath *et al.,* 2009).

The plates were kept at room temperature for half an hour and subsequently incubated at 37 °C and observed for zone of inhibition after 24 hours. The inhibition zone around each disc was measured in millimeter and the assay was carried out three times for each extract. The results were recorded by measuring the zone of growth inhibition surrounding the disc.

3. RESULTS

Eclipta alba is small branched, annual, herbaceous plant with a long history of traditional medicines for many countries, especially tropical and subtropical regions. The herb has been known for its curative properties and utilized as antimytotoxic, analgesic, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, immunemodulatory properties and it is considered as a good rejuvenator too.

The antibacterial activity of the ethanolic extract of whole plant of *Eclipta alba* was studied against both gram positive, *Staphylococcus aureus, Bacillus subtilis* and *Bacillus cereus* and gram negative species, *Escherichia coli, Proteus mirabilis*, *Pseudomonas fluroescens*, *Pseudomonas aeruginosa* and *Salmonella typhi* at 4 different concentrations (1.0, 2.0, 5.0 and 10.0 mg/ml) and the antibacterial activity was compared with that of the standard antibiotic penicillin (10 mg/mL).

The results of antibacterial screening of petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water extracts of *Eclipta alba* are presented in Table 1 and 2. The results revealed variability in inhibitory concentrations of each extract against a given bacteria.

The inhibition of bacterial growth was dose dependent since the inhibitory action of the extract was found to increase with an increase in concentration against all bacterial strains as evidenced by the higher zone of inhibitions at higher concentrations of each extract. Among the various extracts used, acetone extracts of *Eclipta alba* showed the highest activity (zone of inhibition 17.4 mm) against *Proteus mirabilis*.

Acetone extract was appeared to be the most effective extract. None of the water extracts showed any antibacterial activity. None of the chloroform extracts was active against any of the gram positive bacteria tested. The antibacterial activity was more prominent on the gram negative bacteria than the gram positive bacteria. The mean zone of inhibition for the positive control was penicillin.

Table 1. Antibacterial activity of extracts of *Eclipta alba* against Gram positive bacteria.

Extract	Concentration of extract (mg/mL/disc)	Zone of inhibition (mm) Gram positive bacteria		
		Staphylococcus aureus	*Bacillus subtilis*	*Bacillus cereus*
Petroleum ether	1 2 5 10	– 8.0 ± 0.03 10.3±0.05 13.2±0.02	– – – –	– – – –
Chloroform	1 2 5 10	– – – –	– – – –	– – – –
Ethyl acetate	1 2 5 10	8.4±0.04 10.2±0.26 11.6±0.12 13.5±0.12	– – 10.4±0.14 13.8±0.28	– – – –
Acetone	1 2 5 10	- - 7.8±0.03 9.7±0.02	7.4±0.043 10.2±0.39 13.3±0.67 16.9±0.26	- - 10.4±0.44 13.6±0.39
Methanol	1 2 5 10	8.5±0.36 10.6±0.38 13.8±0.02 16.3±0.28	– – 10.7±0.45 12.3±0.32	– – 7.5±0.48 9.7±0.33
Aqueous	1 2 5 10	– – – –	– – – –	– – – –

The results are mean ±SD (n = 8)

Table 2. Antibacterial activity of extracts of *Eclipta alba* against Gram negative bacteria.

Extract	Concentration of extract (mg/mL/disc)	Zone of inhibition (mm) Gram negative bacteria				
		Eschericha coli	*Proteus mirabilis*	*Pseudomonas fluroescens*	*Pseudomonas aeruginosa*	*Salmonella typhi*
Petroleum ether	1 2 5 10	– – 7.6±0.22 9.2±0.26	7.3±9.33 9.6±0.32 12.3±0.57 15.4±0.79	– 9.7±0.56 10.4±0.46 12.6±0.26	– – – –	7.3±0.24 7.7±0.46 8.4±0.29 9.2±0.57

Chloroform	1	–	–	7.2±0.17	–	–
	2	–	–	8.3±0.38	–	–
	5	8.3±0.18	–	12.7±0.36	7.1±0.33	–
	10	11.3±0.57	–	14.5±0.29	7.8±0.24	7.2±0.64
Ethyl acetate	1	9.2±0.54	–	–	–	–
	2	11.2±0.43	–	–	7.2±0.26	–
	5	13.0±0.79	12.2±0.45	8.4±0.59	7.8±0.17	7.1±0.75
	10	16.2±0.24	14.5±0.54	12.3±0.36	8.5±0.58	7.3±0.23
Acetone	1	–	8.3±0.22	7.5±0.68	9.4±0.49	7.6±0.75
	2	–	12.3±0.42	9.6±0.77	9.3±0.39	7.9±0.38
	5	8.2±0.66	14.3±0.53	12.3±0.19	9.8±0.58	8.4±0.52
	10	12.1±0.78	17.4±0.35	14.5±0.33	11.8±0.23	8.8±0.69
Methanol	1	7.3±0.49	8.4±0.22	7.3±0.24	7.2±0.32	–
	2	9.4±0.18	10.5±0.55	9.4±0.44	8.2±0.52	–
	5	12.3±0.31	13.2±0.75	11.6±0.66	8.6±0.22	7.2±0.26
	10	14.0±0.22	16.2±0.82	14.5±0.29	10.6±0.12	7.8±0.14
Aqueous	1	–	–	–	–	–
	2	–	–	–	–	–
	5	–	–	–	–	–
	10	–	–	–	–	–

The results are mean ±SD (n = 8)

4. DISCUSSION

In this study we have demonstrated the antibacterial activity of whole plant extract of *Eclipta alba* against a wide range of various bacterial strains which include gram positive and negative bacteria with the highest antibacterial activity being demonstrated against *Proteus mirabilis*. The antibacterial activity have been screened because of its great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, become an ever increasing therapeutic problem. The presence of antimicrobial substances in higher plants is well established as they provide a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobial compounds. Parallel to increasing the resistance of microorganisms to the currently used antibiotics and the high cost of production of synthetic compounds, pharmaceutical companies are now looking for other alternatives. Continued further research and exploration of plant derived antimicrobials is needed today since such principles represent the vast untapped source for medicine.

Medicinal plants are important source for the development of potential new chemotherapeutic drugs and the *in vitro* antibacterial test form the basis. The broad spectrum antibacterial activities of the plant extract, possibly due to the identified alkaloids, further confirm its use as a health remedy in folklore medicine (Doughari *et al.,*2008). The antibacterial activity of the plants may be due to the presence of various active principles in them. Plant extracts often contains polyphenols and flavonoids which could be the antimicrobial components. The bioactivity of plants extracts is attributed to phytochemical

constituents. Flavonoids are a major group of phenolic compounds reported for their antiviral (Mehrangiz *et al.,*2011), antimicrobial (Mari Lysete *et al.,*2009) and spasmolytic (Julianeli *et al*.,2011) properties. Alkaloids isolated from plants are commonly found to have antimicrobial properties (Ahamed *et al.,* 2010). The antibacterial activities of these compounds might be due to their ability to complex with bacterial cell wall and therefore, inhibiting the microbial growth.

In the present study, the inhibitory action of the extract was found to increase with an increase in concentration against all bacterial strains. Similar results were obtained by different workers in various systems (Khan *et al.,* 2012 and Elumalai *et al.,* 2011)). The inhibitory effect of the extract on the growth of microorganisms could be attributed to the presence of some phytochemicals that were found present in the plant extract. The demonstration of antibacterial activity against both gram positive and gram negative bacteria by this plant may be indicative of the presence of broad spectrum antibiotic compounds (Doughari 2006 and Pandey *et al.,* 2011). The present study justifies the claimed uses of *Eclipta alba* in the traditional system of medicine to treat various infectious diseases caused by the microbes. This study encourages the cultivation of this highly valuable medicinal plant to meet the increasing demand from traditional medicinal system.

5. CONCLUSION

The present investigation showed the effectiveness of crude extract of this plant against tested bacterial strains. This study further suggests the use of whole plant extract in treating diseases caused by tested microbial organisms.

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